IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Sackstein		
SERIAL NUMBER:	10/042,421	EXAMINER:	Phillip Gambel
FILING DATE:	October 18, 2001	ART UNIT:	1644
TITLE:	HEMATOPOIETIC CELL E-SELECTIN/ L-SELECTIN LIGAND POLYPEPTIDES AND METHODS OF USE THEREOF		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. § 1.132

I, Robert Sackstein, declare and state:

1. I am the sole inventor of the subject matter described and claimed in United States Patent Application Serial No. 10/042,421, filed October 18, 2001, entitled "Hematopoietic Cell E-Selectin/ L-Selectin Ligand Polypeptides and Methods of Use Thereof".

2. I understand that the claim of the above-captioned application are directed to purified preparations of CD44 glycoproteins that comprise sialylated, fucosylated glycans and are ligands for E-selectin and/or L-selectin.

3. I have reviewed the Office Action dated August 20, 2009 and have reviewed the references cited therein. I make this declaration to rebut the rejection, with which I do not agree. The rejection set forth in the Office Action is maintained under the belief that "key starting materials" were well known in the art. The discussion and evidence presented in this declaration make clear that the HECA452 monoclonal antibody (mAb) could not be used alone to manufacture the purified preparation of the present claims.

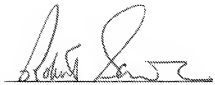
4. It is well known in the field of glycobiology that the monoclonal antibody (mAb) known as HECA452 recognizes various sialylated glycan structures including the tetrasaccharides known as sialylated Lewis X (sLex) and sialylated Lewis A (sLea),

each of which can serve as binding determinants for selectins. These glycan structures can be displayed on protein scaffolds (i.e., glycoproteins) or on lipid scaffolds (i.e., glycolipids). Immunoprecipitation of cell lysates using mAb HECA452 alone would thus yield a complex mixture of molecules - both glycoproteins and glycolipids - some of which could express sLex or sLea or could express various other non-sLea/sLex HECA452-reactive glycan structures found on relevant lipid or protein scaffolds. For this reason, it would not be possible to isolate HCELL from cell lysates by immunoprecipitation or immunoaffinity techniques using HECA452 mAb alone. Notably, our published data provide direct evidence that primitive human hematopoietic cells contain multiple glycoproteins expressing HECA452-reactive determinants. In particular, we published western blot data in 2000 (see Dimitroff et al, *Proceedings of the National Academy of Sciences* 97:13841-13846, 2000) showing that KG1a cells express a variety of HECA452-reactive glycoproteins (see Figure 1A, lanes 4 and 6; see Figure 1B, lanes 1 and 3). Thus, purification of HCELL would never succeed using HECA452 mAb alone.

5. The identification of the HCELL molecule was highly elusive because the binding of L-selectin is shear-dependent, i.e., L-selectin binding to HCELL does not occur under static conditions. Thus, previous attempts at "ligand blotting" using standard selectin probes on western blots of KG1a cell lysates failed to identify HCELL. We thus had to specifically create a totally new technology (the "blot rolling assay") to identify the novel L-selectin ligand (*i.e.*, HCELL) expressed on KG1a cells. This technology was first described in Dimitroff *et al.*, Proceedings of the National Academy of Sciences 97:13841-13846, 2000. The major advantage of this method is that it allows for the rapid and reproducible assessment under appropriate functional shear stress of selectin ligands within a complex mixture of molecules without the need for prior isolation or enrichment of the constituent parts beyond an electrophoretic step and subsequent western blotting. The resultant blotting membrane is rendered semi-transparent by pre-incubation in glycerol-containing buffers, and cells or particles (*e.g.*, microbeads) bearing adhesion molecules of interest (*e.g.*, L-selectin-bearing lymphocytes) are introduced under appropriate physiological flow conditions and observed by video

microscopy for interaction with pertinent immobilized ligands. Tethering and rolling interactions are observed directly on the target ligand band(s). The band(s) supporting such adhesive interactions can then be excised and subjected to mass spectrometry (or protein sequencing) for identification. This inventive step was required to identify the HCELL molecule.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.



Robert Sackstein

1/15/10

Date

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